

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
12 September 2003 (12.09.2003)

PCT

(10) International Publication Number  
WO 03/074521 A1

(51) International Patent Classification<sup>7</sup>: C07D 417/06

(21) International Application Number: PCT/US03/06113

(22) International Filing Date: 28 February 2003 (28.02.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/360,853 1 March 2002 (01.03.2002) US

(71) Applicant (for all designated States except US): UNIVER-  
SITY OF NOTRE DAME [US/US]; 511 Main Building,  
Notre Dame, IN 46556 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): TAYLOR, Richard,  
E. [US/US]; 1228 E Woodside St, South Bend, IN 46614  
(US). CHEN, Yue [CN/US]; 4322 Hickory Rd, Apt 2B,  
Mishawaka, IN 46545 (US).

(74) Agent: JAGTIANI, Ajay; JAGTIANI + GUTTAG,  
10363-A Democracy Lane, Fairfax, VA 22030 (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU,  
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,  
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,  
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,  
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,  
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE,  
SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ,  
VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM,  
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),  
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),  
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,  
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI,  
SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN,  
GQ, GW, ML, MR, NE, SN, TD, TG).

**Declarations under Rule 4.17:**

— as to applicant's entitlement to apply for and be granted  
a patent (Rule 4.17(ii)) for the following designations AE,  
AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH,  
CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI,  
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG,  
KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK,  
MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC,  
SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ,  
VC, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS,  
MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent  
(AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent  
(AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB,  
GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI  
patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML,  
MR, NE, SN, TD, TG)

— as to the applicant's entitlement to claim the priority of the  
earlier application (Rule 4.17(iii)) for all designations

— of inventorship (Rule 4.17(iv)) for US only

**Published:**

— with international search report

For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.

WO 03/074521 A1

(54) Title: DERIVATIVES OF EPOTHILONE B AND D AND SYNTHESIS THEREOF

(57) Abstract: The present invention provides the following new compounds: (R)-C14-methyl-epothilone B, (S)-C14-methyl-epothilone B, (S)-C14-methyl-epothilone D, and (R)-C14-methyl-epothilone D and methods for synthesizing these compounds.

BEST AVAILABLE COPY

## DERIVATIVES OF EPOTHILONE B AND D AND SYNTHESIS THEREOF

### GOVERNMENT INTEREST STATEMENT

5        This invention is made with government support under Grant ID No. CA84599  
awarded by the National Cancer Institute/NIH. The government may have certain rights  
in this invention.

### BACKGROUND OF THE INVENTION

10

#### Field of the Invention

The present invention relates to derivatives of epothilone B and D.

15

#### Description of the Prior Art

Cancer still represents a major unmet medical need. Initial treatment of the  
disease is often surgery, radiation treatment or the combination, but recurrent  
(metastatic) disease is common. Chemotherapeutic treatments for most cancers are  
20 generally not curative, but only delay disease progression. Commonly, tumors and their  
metastases become refractory to chemotherapy, in an event known as development of  
multidrug resistance. In many cases, tumors are inherently resistant to some classes of  
chemotherapeutic agents.

25

Among cytotoxic agents for the treatment of tumors, TAXOL® (paclitaxel), a  
microtubule stabilizing agent, has become a very important compound with a  
remarkable economic success. However, TAXOL® has a number of disadvantages.  
Especially its extremely low solubility in water represents a severe problem. It has  
become necessary to administer TAXOL® in a formulation with Cremophor EL®  
30 (polyoxyethylated castor oil; BASF, Ludwigshafen, Germany) which has severe side

effects, causing inter alia allergic reactions that in one case even were reported to have led to the death of a patient. More severely, certain tumor types are known to be refractory to treatment with TAXOL® even when the drug is administered as front-line therapy, or the tumors develop resistance to TAXOL® after multiple cycles of exposure.

5

Although the taxane class of antimicrotubule anti-cancer agents has been hailed as the perhaps most important addition to the chemotherapeutic armamentarium against cancer over the past several decades and despite the commercial success of TAXOL®, there remain limitations to TAXOL®'s efficacy. TAXOL® treatment is associated with a number of significant side effects and some major classes of solid tumors, namely colon and prostate, are poorly responsive to this compound. For example, the effectiveness of TAXOL® can be severely limited by acquired drug resistance mechanisms occurring via various mechanisms, such as overexpression of phosphoglycoproteins that function as drug efflux pumps.

15

Therefore, there exists an urgent need to find compounds and appropriate dosing regimens with these compounds to expand the armamentarium of cancer treatment, especially in the majority of cases where treatment with taxanes and other anticancer compounds is not associated with long term survival.

20

### SUMMARY OF THE INVENTION

It is therefore an object of the present invention to provide new compounds that may be used effectively in cancer chemotherapy.

25

It is a further object of the present invention to provide compounds that may be used to treat TAXOL®-resistant tumors.

30

It is yet a further object of the present invention to provide new derivatives of epothilone B.

It is yet a further object of the present invention to provide new derivatives of epothilone D.

5 According to a first broad aspect of the present invention, there is provided compound comprising (*R*)-C14-methyl-epothilone B.

According to second broad aspect of the present invention, there is provided a compound comprising (*S*)-C14-methyl-epothilone B.

10

According to third broad aspect of the present invention, there is provided a compound comprising (*S*)-C14-methyl-epothilone D.

15

According to fourth broad aspect of the present invention, there is provided a compound comprising (*R*)-C14-methyl-epothilone D

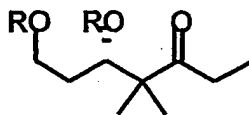
According to a fifth broad aspect of the present invention, there is provided a method for synthesizing (*R*)-C14-methyl-epothilone B comprising the steps of: providing (*S*)-C14-methyl-epothilone D; and (b) incorporating an epoxide group at the C12-C14 position of (*S*)-C14-methyl-epothilone D to form (*R*)-C14-methyl-epothilone B.

20

According to a sixth broad aspect of the invention, there is provided a method for synthesizing (*S*)-C14-methyl-epothilone D comprising the following steps:

25

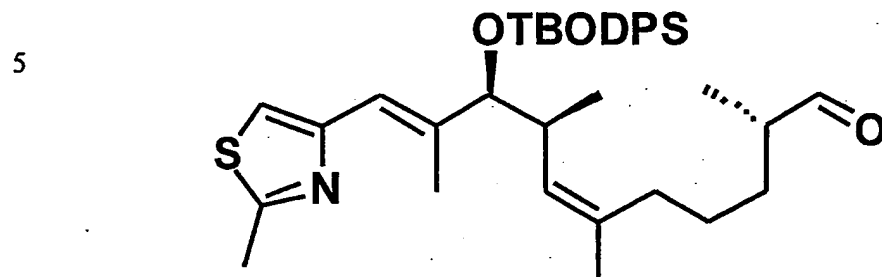
(a) providing an ethyl ketone 10 having the following formula:



30

(b) exposing ethyl ketone 10 to LDA to produce the lithium enolate of ethyl ketone 10;

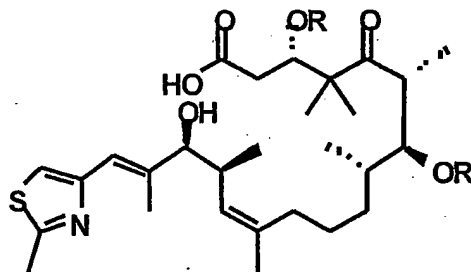
(c) mixing ethyl ketone 10 with an aldehyde 9 having the following formula:



10

to thereby form a *syn*, *anti*-aldol adduct having a primary alcohol and a C7 hydroxyl;  
 (d) exposing said *syn*, *anti*-aldol adduct to TBSOTf to convert said C7 hydroxyl to a TBS ether; (e) de-protecting said *syn*, *anti*-aldol adduct to form a primary alcohol; (f) oxidizing said primary alcohol to form an oxidized adduct having a C1-carboxylic acid;  
 15 (g) removing a C15 TBODPS ether from said oxidized adduct to form a compound 11 having the following formula:

20



(h) macrolactonizing compound 11 to form a 16-membered lactone; and (i) treating said  
 25 16-membered lactone with TFA to form (*S*)-C14-methyl-epothilone D.

Other objects and features of the present invention will be apparent from the following detailed description of the preferred embodiment.

30

### **BRIEF DESCRIPTION OF THE DRAWINGS**

The invention will be described in conjunction with the accompanying drawings,  
in which:

5

FIG. 1 illustrates two conformations of epothilone B and two derivatives of  
epothilone B;

FIG. 2 illustrates a first scheme of a method of the present invention for forming  
10 first intermediates useful in synthesizing the epothilone B and D derivatives of the  
present invention;

FIG. 3 illustrates a second scheme of a method of the present invention, using  
the first intermediates of Figure 2, for forming second intermediates useful in  
15 synthesizing the epothilone B and D derivatives of the present invention; and

FIG. 4 illustrates a third scheme of a method of the present invention, using the  
second intermediates of Figure 3 to form the epothilone B and D derivatives of the  
present invention; and

20

FIG. 5 illustrates a method of the present invention for forming an epothilone B  
derivative of the present invention from an epothilone D derivative of the present  
invention.

25

### **DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT**

It is advantageous to define several terms before describing the invention. It  
should be appreciated that the following definitions are used throughout this application.

### Definitions and Abbreviations

For the purposes of the present invention, the term "scheme" refers to one or more sub-steps of a method for synthesizing an epothilone derivative of the present invention.

5

The following abbreviations appear in Schemes 1, 2 and 3 of FIGS. 2, 3 and 4, respectively: Z-crotyl-(+)-Ipc<sub>2</sub>B (Z-3-methylpropenyl-diisopinocampheyl borane), described in Brown, H. C. and Bhat, K. S. "Chiral Synthesis via Organoboranes. 7. Diastereoselective and Enantioselective Synthesis of *Erythro*- and  
10 *Threo*-β-methylhomoallyl Alcohols via Enantiomeric (Z)- and (E)-Crotylboranes" *J. Am. Chem. Soc.* 1986, 108, 5919-5923, the entire contents and disclosure of which is hereby incorporated by reference), NMO (4-methylmorpholine N-oxide) LDA (lithium diamine), TBODPSCI (*t*-butoxydiphenylsilyl chloride), TBSOTf (*t*-butyl-dimethylsilyltriflate) TBS (*t*-butyl-dimethylsilyl), CSA (camphorsulfonic acid), DMP  
15 (Dess-Martin periodinane, described in Dess D. B.; Martin J. C. "Readily Accessible 12-I-5 Oxidant for the Conversion of Primary and Secondary Alcohols to Aldehydes and Ketones" *J. Org. Chem.* 1983, 48, 4155-4156, the entire contents and disclosure of which is hereby incorporated by reference), TBAF (tetrabutylammonium fluoride, TCBCl (2,4,5-trichlorobenzoyl chloride), DIEA (N,N-diisopropylethylamine), DMAP  
20 (4-(dimethylamino)pyridine), TFA (trifluoroacetic acid), and mCPBA (m-Chloroperoxy benzoic acid).

### Description

25 The success of the anticancer drug Taxol®, marketed by Bristol-Myers Squibb, appears to be related to its unique mode of action, tubulin polymerization and microtubule stabilization. However, Taxol®'s low water solubility and inactivity against multi-drug resistant tumors limits Taxol®'s usefulness. Compounds with biological activity related to Taxol®'s activity included the epothilones, but the known epothilones  
30 and epothilone derivatives are not satisfactory for all purposes. Several years ago, a study by Taylor *et al.* on the conformational properties of the epothilones which utilized

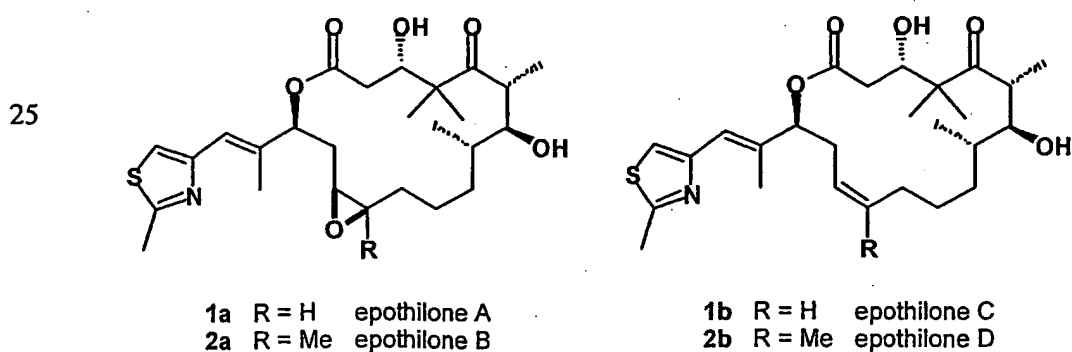
a combination of computational methods and high field NMR experiments, see Taylor, R. E. and Zajicek, J. "The Conformational Properties of Epothilone" *J. Org. Chem.* 1999, 64, 7224. The Taylor *et al.* study concentrated on the critical C1-C8 polypropionate region and concluded that in solution the epothilones preferred to exist in at least two conformational families controlled by the *syn*-pentane interactions. In addition, the Taylor *et al.* study showed that the major contributor was indeed related to the conformation observed in the solid state, see also Hoefle, G.; Bedorf, N.; Steinmetz, H.; Schomburg, D.; Gerth, K.; and Reichenbach, H. "Epothilone A and B—Novel 16-membered Macrolides with Cytotoxic Activity: Isolation, Crystal Structure, and Conformation in Solution" *Angew. Chemie* 1996, 35, 1567-1569. The conversion of an allylic alcohol to an allylic chloride useful in synthesizing epothilone derivatives is described in Taylor, R. E.; and Chen, Y. "The Total Synthesis of Epothilones B and D" *Organic Lett.* 2001, 3, 2221, the entire contents and disclosure of which is hereby incorporated by reference.

15

The present invention provides epothilone derivatives and method of making epothilone derivatives based on the stabilization of conformational families through simple substitution. Several of the preferred epothilone derivatives of the present invention have significant biological activity and together provide new insights into the biological active conformation of the epothilone class of natural products.

20

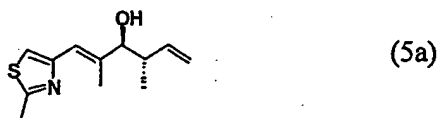
The chemical formulas for epothilones A, B, C and D are shown below:



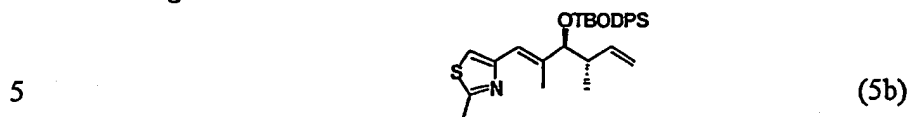


With respect to the C10-C15 epoxide region of epothilone B and epothilone B derivatives, a high degree of flexibility leading to two conformational families shown as Conformer A and B, in which R = H for epothilone B and R = Me for the epothilone B derivative 3 ((R)-C14-methyl epothilone B) and epothilone B derivative 4 ((S)-C14-methyl epothilone B) of FIG. 1. The epothilone B derivatives 3 and 4 are intriguing because epothilone B derivatives 3 and 4 represent an acetate to propionate modification in the biogenetic pathway. Therefore, advances in genetic engineering of polyketide synthases, see Khosla, C. "Natural Product Biosynthesis: A New Interface between Enzymology and Medicine" J. Org. Chem. 2000, 65, 8127-8133, may provide alternative methods, in addition to the chemical synthesis methods described below, for producing epothilone B derivative 3 through manipulation of the epothilone gene cluster, see Julien, B.; Shah, S.; Ziermann, R.; Goldman, R.; Katz, L.; and Khosla, C. "Isolation and Characterization of the Epothilone Biosynthetic Gene Cluster from *Sorangium cellulosum*" *Gene* 2000, 249, 153-160. b) Tang, L.; Shah, S.; Chung, L.; Carney, J.; Katz, L.; Khosla, C.; and Julien, B. *Science* 2000, 287, 640-642. c) Molnar, I.; Schupp, T.; Ono, M.; Zirkle, R.; Milnamow, M.; Nowak-Thompson, B.; Engel, N.; Toupet, C.; Stratmann, A.; Cyr, D. D.; Gorlach, J.; Mayo, J. M.; Hu, A.; Goff, S.; Schmid, J.; and Ligon, J. M. "The Biosynthetic Gene Cluster for the Microtubule-Stabilizing Agents Epothilones A and B from *Sorangium cellulosum* So ce90" *Chem. Biol.* 2000, 7, 97-109, or through semi-synthesis/biosynthetic techniques used on *Sorangium cellulosum*, the organism from which epothilones are generally produced.

In a preferred method of the present invention, (S)-C14-methyl-epothilone D is synthesized using thiazole aldehyde 5 of Scheme 1 of FIG. 2. A brown asymmetric crotylboration using Z-crotyl-(+)-Ipc<sub>2</sub>B to form compound 5a having the following formula:

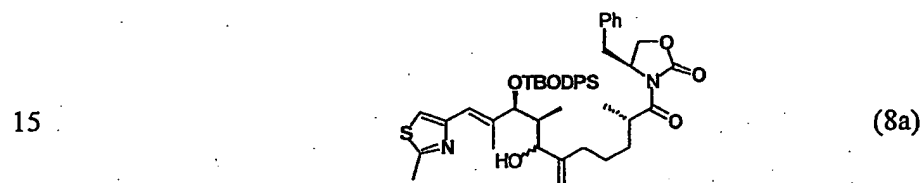


Compound 5a is treated with TBODPSCl to protect the secondary hydroxyl of Compound 5a as t-butoxydiphenylsilyl ether and form compound 5b having the following formula:

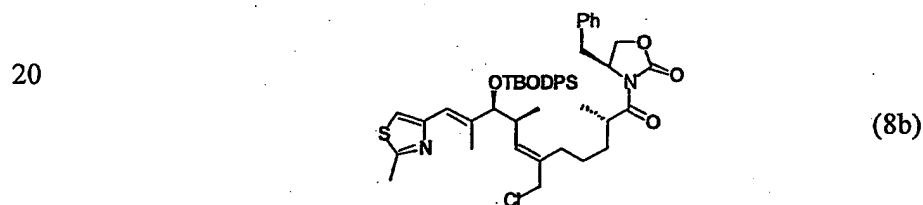


An oxidative cleavage of the terminal alkene using OsO<sub>4</sub>, NMO followed by NaIO<sub>4</sub> may be performed on compound 5b to form aldehyde 6 of Scheme 1.

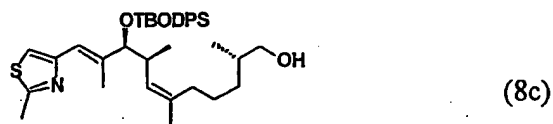
10 Next, a vinyl aldehyde 8, shown in Scheme 2 of FIG. 3, is intermolecularly coupled with aldehyde 6 using Ni/Cr coupling to provide an intermediate allylic alcohol 8a having the following formula as a mixture of diastereomers (1:1). :



Exposure of this mixture to thionyl chloride in ether-pentane provides a primary allylic chloride 8b having the following formula:

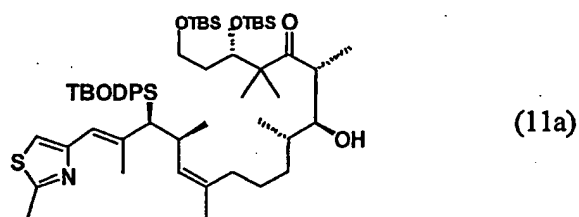


25 Reacting the primary allylic chloride 8b with LiEt<sub>3</sub>BH form a primary alcohol 8c having the following formula:



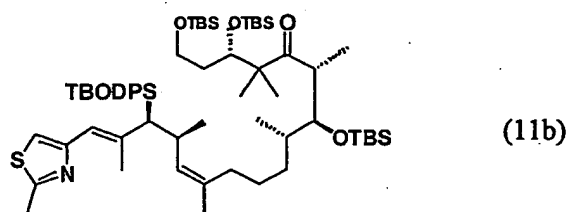
30 Primary alcohol 8c is then oxidized with Dess-Martin periodane to provide aldehyde 9 of Scheme 2.

Next, ethyl ketone 10 shown in Scheme 3 of FIG. 4 is reacted with LDA and aldehyde 9 to form a *syn, anti*-aldol adduct 11a having the following formula:

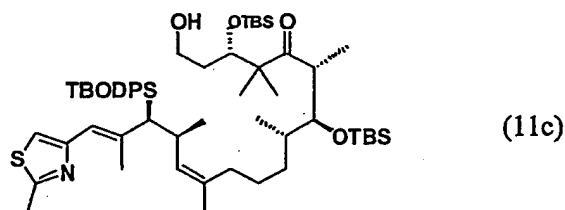


The C7 hydroxyl of *syn, anti*-aldol adduct 11a is protected as a TBS ether by reacting *syn, anti*-aldol adduct 11a with TBSOTf to form an etherized adduct 11b having the following formula:

10

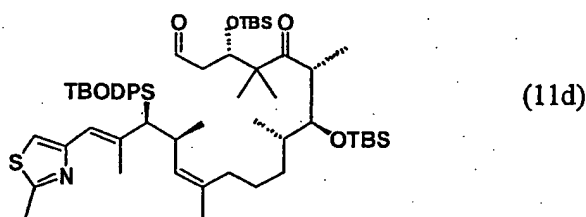


Etherized adduct 11b is exposed to acidic methanol solution to liberate a primary alcohol 11c having the following formula:

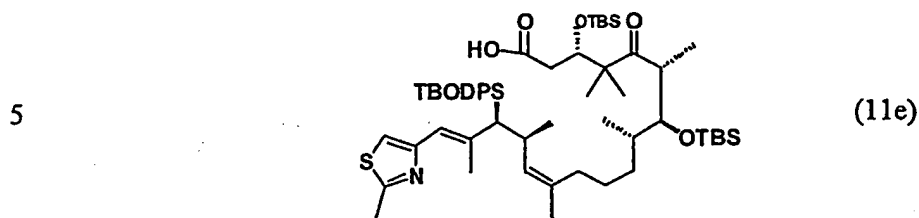


Primary alcohol 11c is treated with DMP to provide aldehyde 11d having the following formula:

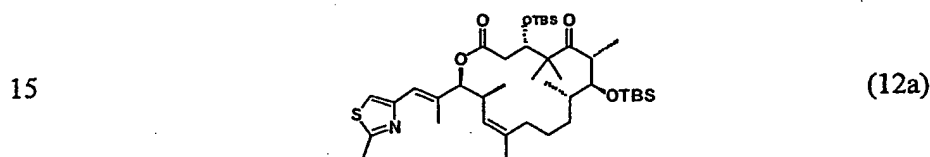
25



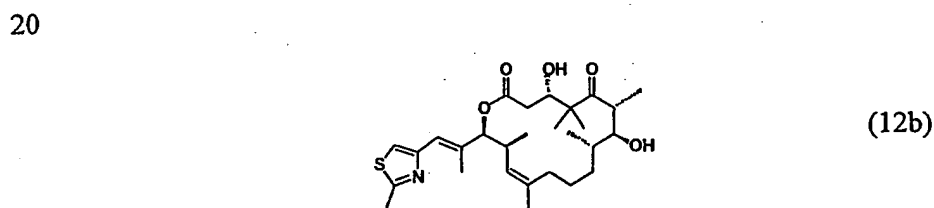
Aldehyde 11d is then oxidized to a carboxylic acid, oxidized adduct 11e having the following formula:



Oxidized adduct 11e is then treated with TBAF to remove the C15 TBSDPS ether to form alcohol 11 of Scheme 3 of FIG. 4. A macrolactonization is then carried out on alcohol 11 using TCBCl, DIEA and DMAP to form a 16-membered lactone 12a having the following formula:



A deprotection of the C3 and C7 silyl ethers of lactone 12a is then carried out using TFA to form (S)-C14-methyl epothilone D 12b having the following formula:



25 In a preferred method of the present invention, (R)-C14-methyl-epothilone B is synthesized by exposing (S)-C14-methyl-epothilone D to mCPBA.

A method similar to the method described above for synthesizing (S)-C14-methyl-epothilone D from thiazole aldehyde 5 may be used to synthesize (R)-C14-methyl-epothilone D by carrying out the crotylboration of thiazole aldehyde 5 with E-crotyl-(+)-Ipc<sub>2</sub>B instead of Z-crotyl-(+)-Ipc<sub>2</sub>B to form aldehyde 7 instead of aldehyde 6.

The remaining synthesis steps to form (*R*)-C14-methyl-epothilone D from aldehyde 7 are similar to the synthesis steps used to form (*S*)-C14-methyl-epothilone D from aldehyde 6.

- 5 In a preferred method of the present invention, (*S*)-C14-methyl-epothilone B may be synthesized by exposing (*R*)-C14-methyl-epothilone D to mCPBA.

## EXAMPLES

### 10 Example 1

(*R*)-C14-methyl epothilone B (epothilone B derivative 3) was synthesized using a method shown in Schemes 1, 2 and 3 of FIGS. 2, 3 and 4, respectively.

- 15 Readily available thiazole aldehyde 5, a common intermediate in several synthetic routes to these epothilone, is the point divergence for the synthesis of epothilone B derivatives 3 and 4 as shown in Scheme 1. Brown asymmetric crotylboration<sup>9</sup> efficiently controlled the enantioselectivity as well as the diastereoselectivity of the C14, 15 stereogenic centers. Protection of the secondary  
20 hydroxyl as a *t*-butoxydiphenylsilyl ether followed by oxidative cleavage of the terminal alkene provided aldehydes 6 and 7.

- The conversion of aldehydes 6 and 7 to epothilone B derivatives 3 and 4, respectively, proceeded through identical synthetic sequences. Only the synthesis of  
25 epothilone B derivative 3 from aldehyde 6 is described below and shown in Schemes 2 and 3, of FIGS. 3 and 4, respectively. However, (*S*)-14-methyl epothilone B (epothilone B derivative 4) may be synthesized in a similar manner by substituting aldehyde 7 for aldehyde 6 in the first step of Scheme 2 of FIG. 3.

- 30 As shown in Scheme 2 of FIG. 3, intermolecular Ni/Cr coupling of vinyl iodide 8 with aldehyde 6 (2 equiv.) provided the intermediate allylic alcohol in 75% yield as a

mixture of diastereomers (1:1). Exposure of this mixture to thionyl chloride in ether-pentane provided the desired primary chloride in 85% yield. While the olefin geometry was completely selective a small amount of the secondary allylic chloride could be observed in the crude proton NMR spectrum. LiEt<sub>3</sub>BH not only reductively cleaved the chiral auxiliary but also efficiently reduced the allylic chloride in 72% yield providing primary alcohol 8c. Oxidation with Dess-Martin periodinane then provided aldehyde 9.

As highlighted in Scheme 3 of FIG. 4, the completion of the total synthesis began with an aldol reaction. The lithium enolate of ethyl ketone 10 was generated by exposure to LDA at -78 °C. Addition of aldehyde 9 provided the desired *syn, anti*-aldol adduct as the major product in 69% yield and 3.5:1 selectivity. After protection of the C7 hydroxyl as a TBS ether the primary alcohol was liberated by exposure to acidic methanol solution. The C1-carboxylic acid 11 was generated by two-step oxidation and subsequent selective removal of the C15 TBODPS (*t*-butoxydiphenylsilyl) ether was then accomplished with TBAF in a combined 71% yield. Macrolactonization proceeded efficiently using the Yamaguchi method<sup>12</sup> to provide the 16-membered lactone in 78% yield. Deprotection of the C3 and C7 silyl ethers was carried out using TFA providing the (*S*)-C14-methyl epothilone D 12. Finally, a highly selective incorporation of the C12-C13 epoxide was carried out by exposure to mCPBA yielding (*R*)-C14-methyl epothilone B in 55% yield.

Synthesis of (*R*)-C14-methyl epothilone D, epothilone D derivative 13 was accomplished via a similar synthetic sequence to that described for the synthesis of (*R*)-C14-methyl epothilone D. Epoxidation of this intermediate proceeded in a highly selective fashion as shown in Scheme 4 of FIG. 5. The conformational constraints imposed by the C14-methyl substituent makes it highly likely that the stereochemistry of the epoxide is epimeric to epothilone B and epothilone derivatives 3 and 4. The resulting epothilone derivative appears to have the structure shown at 14.

Single crystals of epothilone D derivative 12 and epothilone B derivative 3 were obtained by slow-evaporation techniques and x-ray diffraction studies unambiguously

determined their structure, Figure 2. In addition, the conformation of each in the solid-state was quite similar to that reported for epothilone B and thus conformer A. Proton NMR coupling constants ( $J_{14-15} = 9.9, 10.5$  Hz respectively) also supported this preference in solution. In contrast, proton NMR coupling constants of epothilone derivatives 13 and 14 had the expected values for conformer B ( $J_{14-15} = 2.9, 1.2$  Hz respectively).

Preliminary biological investigations of these compounds revealed significant tubulin polymerization activity for compounds 12 and 3. In contrast, the C14-diastereomeric compounds, epothilone derivatives 13 and 14, showed relatively weak activity. Similar profiles were observed in tumor cell growth assays. In fact, (*R*)-C14-methyl-epothilone B 3 was found to be >2x as active as epothilone B itself.

The conformation-activity relationships presented herein strongly support the importance of conformer A for the tubulin binding. Moreover, this approach represents on new perspective on rational design of new chemotherapeutic agents.

The synthesis methods described above for epothilone derivatives 3 and 4 was highlighted by the efficient generation of a C12-C13 trisubstituted olefin which exploits a sequential Nozaki-Hiyama-Kishi coupling and a stereoselective thionyl chloride rearrangement. Using the above-described synthesis method, quantities of epothilone derivatives 3 and 4 may be produced.

## Example 2

### **Materials and Methods**

*Cell lines and culture conditions:* Human breast carcinoma cell line MCF-7, multi-drug resistant breast carcinoma cell line NCI/ADR, non-small cell lung carcinoma cell line NCI-H460 and glioma cell line SF-268 were obtained from the National Cancer Institute. All cell lines were maintained in RPMI-1640 medium (Gibco/BRL, Rockville,

MD) supplemented with 2 mM L-glutamine, 25 mM HEPES and 10% FBS (Hyclone, Logan, UT). Cells were maintained in a humidified incubator at 37°C in 5% CO<sub>2</sub>.

*Cytotoxicity assays:* Tumor cells were seeded in 100  $\mu$ l at 5000 (MCF-7), 7500 (NCI/ADR), 5000 (NCI-H460) and 7500 (SF-268) cells per well in 96-well plates. Cells were allowed to adhere for 24 hours. Each compound ranging from 0.001 to 1000 nM in 100  $\mu$ l was added to cells in triplicate wells. After 3 days, cells were fixed at 4°C for 1 hour with 10% trichloroacetic acid and then stained with 0.2% sulforhodamine B (SRB)/1% acetic acid for 20 minutes at room temperature. The unbounded dye was rinsed away with 1% acetic acid, and the bounded SRB was then extracted with 200  $\mu$ l of 10 mM Tris base. The absorbance was measured at 515 nm using a 96-well microtiter plate reader (Spectra Max 250, Molecular Devices). The IC<sub>50</sub> values were calculated using a KaleidaGraph program. The experiments were performed twice.

## 15 Results

IC<sub>50</sub> (nM) Values

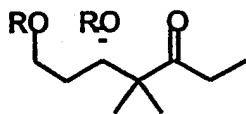
| Compound                | MCF-7 | NCI-ADR | H460  | SF    |
|-------------------------|-------|---------|-------|-------|
| Epothilone D            | 5     | 26      | 20    | 7     |
| 14s-methyl Epothilone D | 35    | 238     | 42    | 42    |
| 14r-methyl Epothilone B | 3     | 23      | 3     | 3     |
| 14r-methyl Epothilone D | >1000 | >1000   | >1000 | >1000 |
| 14s-methyl Epothilone B | >1000 | >1000   | >1000 | >1000 |

Although the present invention has been fully described in conjunction with the preferred embodiment thereof with reference to the accompanying drawings, it is to be understood that various changes and modifications may be apparent to those skilled in the art. Such changes and modifications are to be understood as included within the scope of the present invention as defined by the appended claims, unless they depart therefrom.



**WHAT IS CLAIMED IS:**

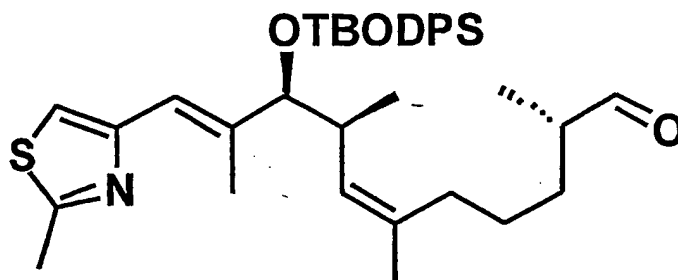
1. A compound comprising (*R*)-C14-methyl-epothilone B.
2. A compound comprising (*S*)-C14-methyl-epothilone B.
3. A compound comprising (*S*)-C14-methyl-epothilone D.
4. A compound comprising (*R*)-C14-methyl-epothilone D.
5. A method for synthesizing (*R*)-C14-methyl-epothilone B comprising the steps of:
  - (a) providing (*S*)-C14-methyl-epothilone D; and
  - (b) incorporating an epoxide group at the C12-C14 position of (*S*)-C14-methyl-epothilone D to form (*R*)-C14-methyl-epothilone B.
6. The method of claim 5, wherein step (b) is carried out by exposing (*S*)-C14-methyl-epothilone D to mCPBA.
7. The method of claim 5, wherein (*S*)-C14-methyl-epothilone D is provided synthesizing (*S*)-C14-methyl-epothilone D by a method comprising the following steps:
  - (c) providing an ethyl ketone 10 having the following formula:



;

- (d) exposing ethyl ketone 10 to LDA to produce the lithium enolate of ethyl ketone 10;

(e) mixing ethyl ketone 10 with an aldehyde 9 having the following formula:



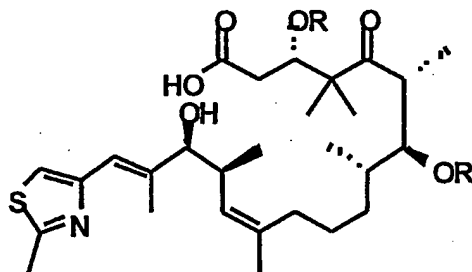
to thereby form a *syn*, *anti*-aldol adduct having a primary alcohol and a C7 hydroxyl;

(f) exposing said *syn*, *anti*-aldol adduct to TBSOTf to convert said C7 hydroxyl to a TBS ether;

(g) de-protecting said *syn*, *anti*-aldol adduct to form a primary alcohol;

(h) oxidizing said primary alcohol to form an oxidized adduct having a C1-carboxylic acid;

(i) removing a C15 TBODPS ether from said oxidized adduct to form a compound 11 having the following formula:



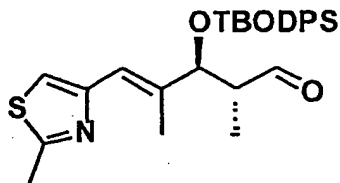
;

(j) macrolactonizing compound 11 to form a 16-membered lactone; and

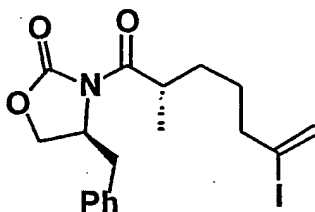
(k) treating said 16-membered lactone with TFA to form (*S*)-C14-methyl-epothilone D.

7. The method of claim 6, wherein aldehyde 9 is synthesized by a method comprising the following steps:

(l) providing aldehyde 6 having the following formula:



(m) intermolecularly coupling aldehyde 6 with a vinyl iodide 8 having the following formula:



using Ni/Cr coupling to thereby form an intermediate allylic alcohol;

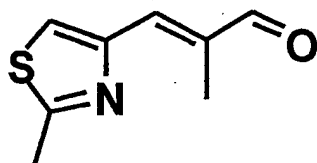
(n) reacting said allylic alcohol thionyl chloride to produce a primary allylic chloride;

(o) reacting said primary allylic chloride with  $\text{LiEt}_3\text{BH}$  to form a primary allylic alcohol; and

(p) oxidizing said primary allylic alcohol to form aldehyde 9.

8. The method of claim 7, wherein aldehyde 6 is provided by synthesizing aldehyde 6 using a method comprising the following steps:

(q) providing thiazole aldehyde 5 having the following formula:



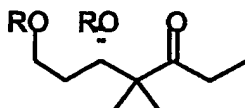
(r) reacting thiazole aldehyde 5 with Z-crotyl(+)-Ipc<sub>2</sub>B to form a homoallylic alcohol compound;

(s) reacting said homoallylic alcohol with TBODPSCl to form an etherized compound; and

(t) oxidatively cleaving a terminal alkene of said etherized compound to form aldehyde 6.

9. A method for synthesizing (S)-C14-methyl-epothilone D comprising the following steps:

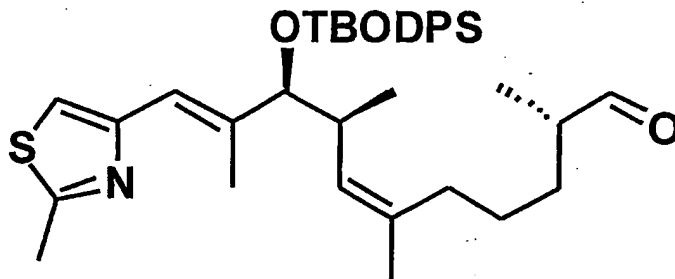
(a) providing an ethyl ketone 10 having the following formula:



;

(b) exposing ethyl ketone 10 to LDA to produce the lithium enolate of ethyl ketone 10;

(c) mixing ethyl ketone 10 with an aldehyde 9 having the following formula:



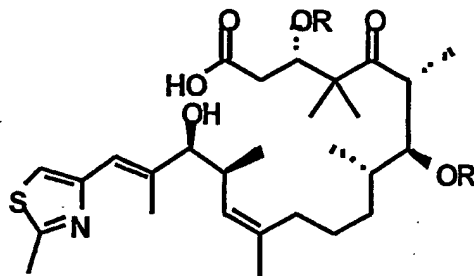
to thereby form a *syn*, *anti*-aldol adduct having a primary alcohol and a C7 hydroxyl;

(d) exposing said *syn*, *anti*-aldol adduct to TBSOTf to convert said C7 hydroxyl to a TBS ether;

(e) de-protecting said *syn*, *anti*-aldol adduct to form a primary alcohol;

(f) oxidizing said primary alcohol to form an oxidized adduct having a C1-carboxylic acid;

(g) removing a C15 TBODPS ether from said oxidized adduct to form a compound 11 having the following formula:

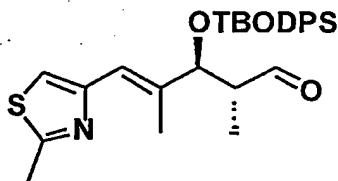


(h) macrolactonizing compound 11 to form a 16-membered lactone; and

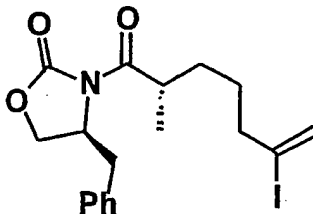
(i) treating said 16-membered lactone with TFA to form (S)-C14-methyl-epothilone D.

10. The method of claim 9, wherein aldehyde 9 is synthesized by a method comprising the following steps:

(j) providing aldehyde 6 having the following formula:



(k) intermolecularly coupling aldehyde 6 with a vinyl iodide 8 having the following formula:



using Ni/Cr coupling to thereby form an intermediate allylic alcohol;

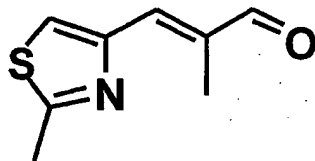
(l) reacting said allylic alcohol thionyl chloride to produce a primary allylic chloride;

(m) reacting said primary allylic chloride with  $\text{LiEt}_3\text{BH}$  to form a primary allylic alcohol; and

(n) oxidizing said primary allylic alcohol to form aldehyde 9.

11. The method of claim 10, wherein aldehyde 6 is provided by synthesizing aldehyde 6 using a method comprising the following steps:

(o) providing thiazole aldehyde 5 having the following formula:



;

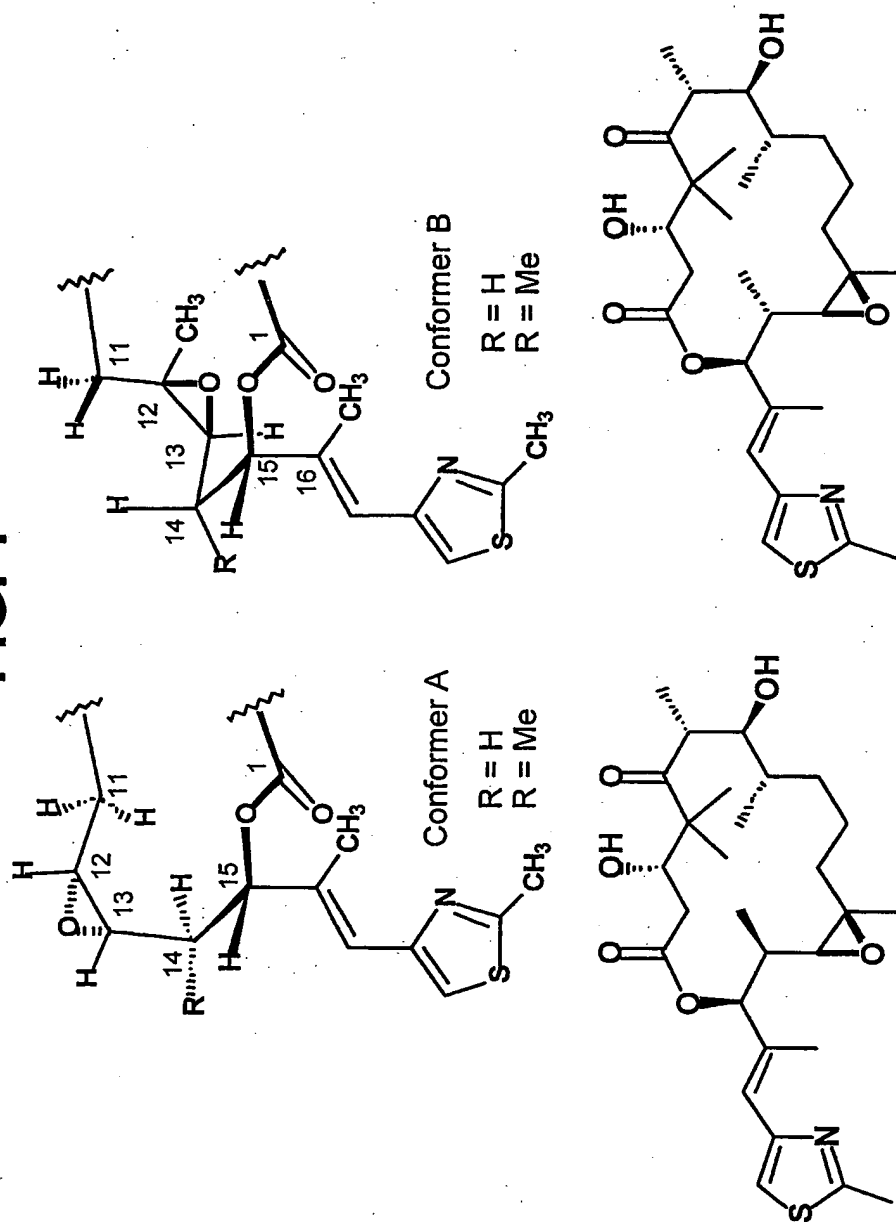
(q) reacting thiazole aldehyde 5 with Z-crotyl(+)- $\text{Ipc}_2\text{B}$  to form a chloroborated compound;

(r) reacting said chloroborated aldehyde with TBODPSCl to form an etherized compound; and

(s) oxidatively cleaving a terminal alkene of said etherized compound to form aldehyde 6.

1/5

FIG. 1



2/5

## FIG. 2

## Scheme 1

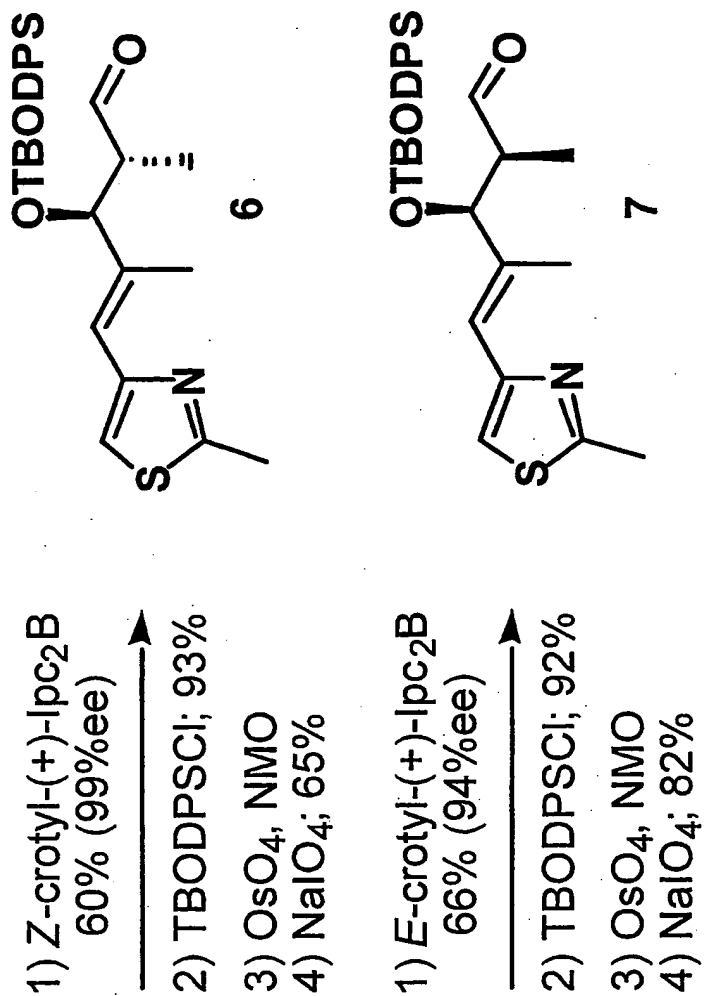
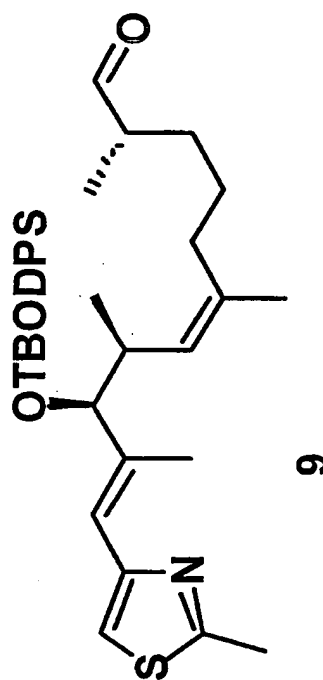
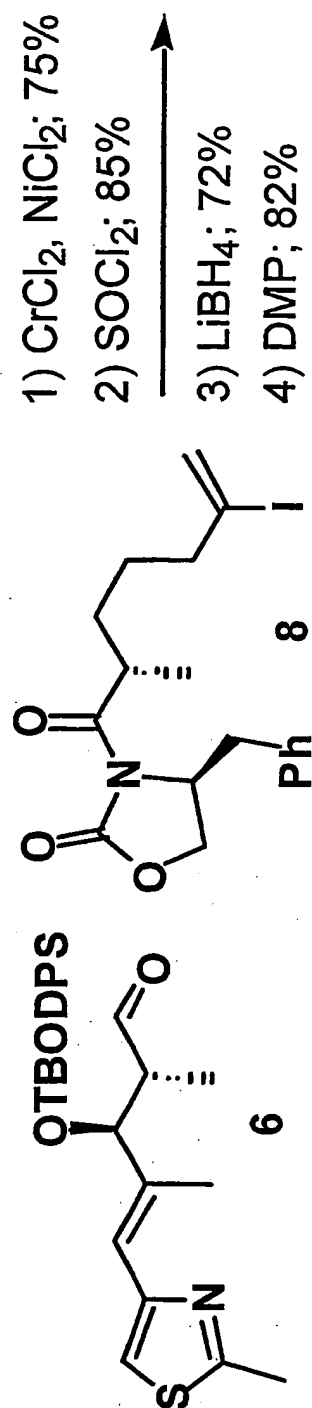




FIG. 3

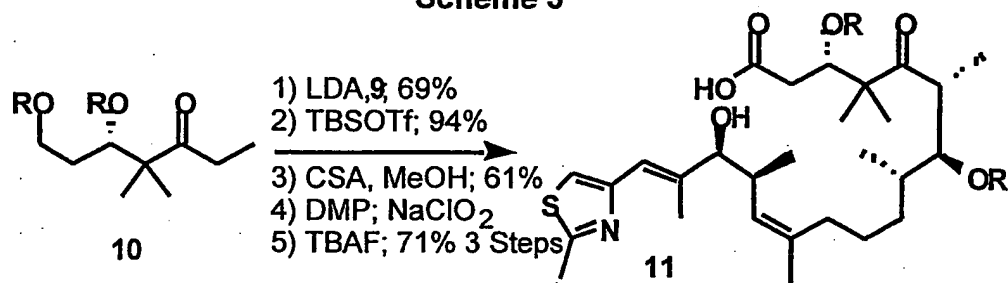
Scheme 2



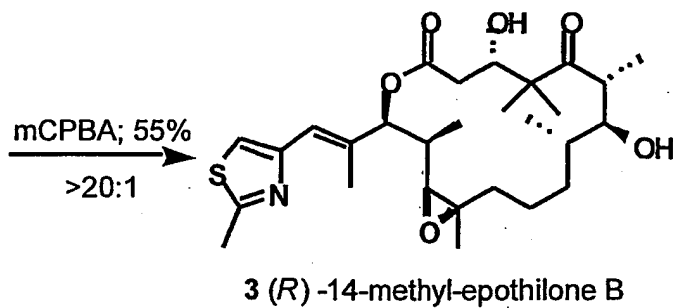
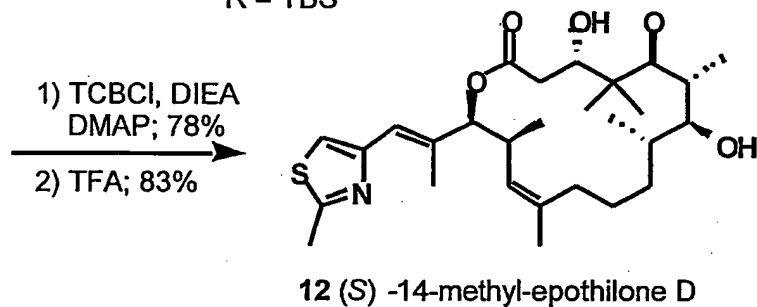
4/5

## FIG. 4

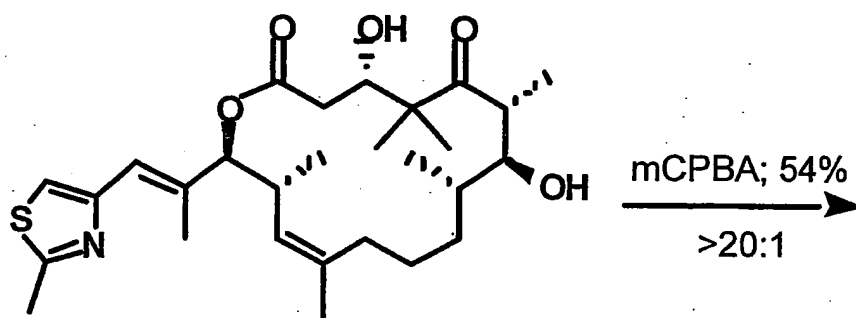
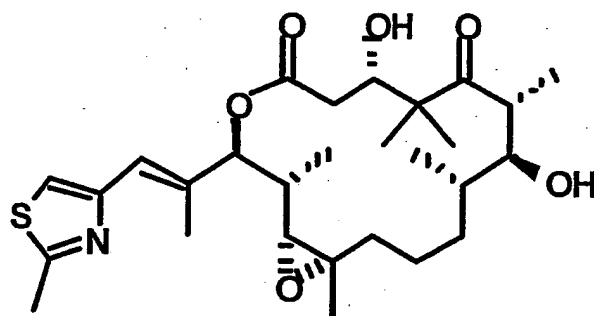
Scheme 3



R = TBS



5/5

**FIG. 5****Scheme 4****13 (R) -14-methyl-epothilone D****14 (S) -14-methyl analogue**

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/06113

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) : CO7D 417/06

US CL : 548/204

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**Minimum documentation searched (classification system followed by classification symbols)  
U.S. : 548/204

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
STN**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category * | Citation of document, with indication, where appropriate, of the relevant passages       | Relevant to claim No. |
|------------|--|-----------------------|
| X          | WO 01/083800 A2 (KOSAN BIOSCIENCES, INC.) 8 November 2001 (08.11.2001), see example 11G. | 1-6                   |

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

| Special categories of cited documents:  |  |
|---|--|
| "A" document defining the general state of the art which is not considered to be of particular relevance  | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention  |
| "E" earlier application or patent published on or after the international filing date   | "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone   |
| "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "O" document referring to an oral disclosure, use, exhibition or other means  | "&" document member of the same patent family  |
| "P" document published prior to the international filing date but later than the priority date claimed  |  |

Date of the actual completion of the international search

19 May 2003 (19.05.2003)

Date of mailing of the international search report

02 JUN 2003

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, Virginia 22313-1450

Facsimile No. (703)305-3230

Authorized officer

Robert Gerstl  
D. Roberts for

Telephone No. 703 308-1235

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☒ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**